

SUPPLEMENTAL INFORMATION

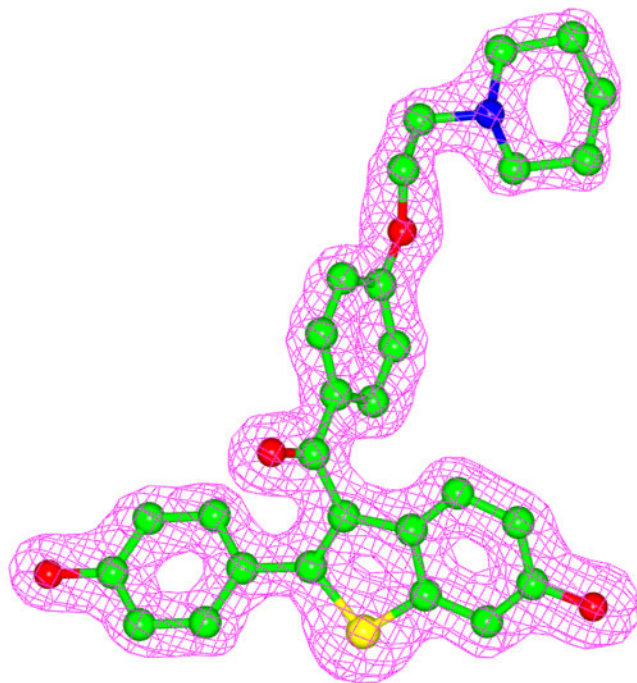
NF κ B selectivity of estrogen receptor ligands revealed by comparative crystallographic analyses

Kendall W. Nettles, John B. Bruning, German Gil, Jason Nowak, Sanjay K. Sharma, Johnnie B. Hahm, Kristen Kulp, Richard B. Hochberg, Haibing Zhou, John A. Katzenellenbogen, Benita S. Katzenellenbogen, Yunchang Kim, Andrzej Joachim, Geoffrey L. Greene

Correspondence:

Kendall Nettles, Tel 561-799-8851, knettlles@scripps.edu;

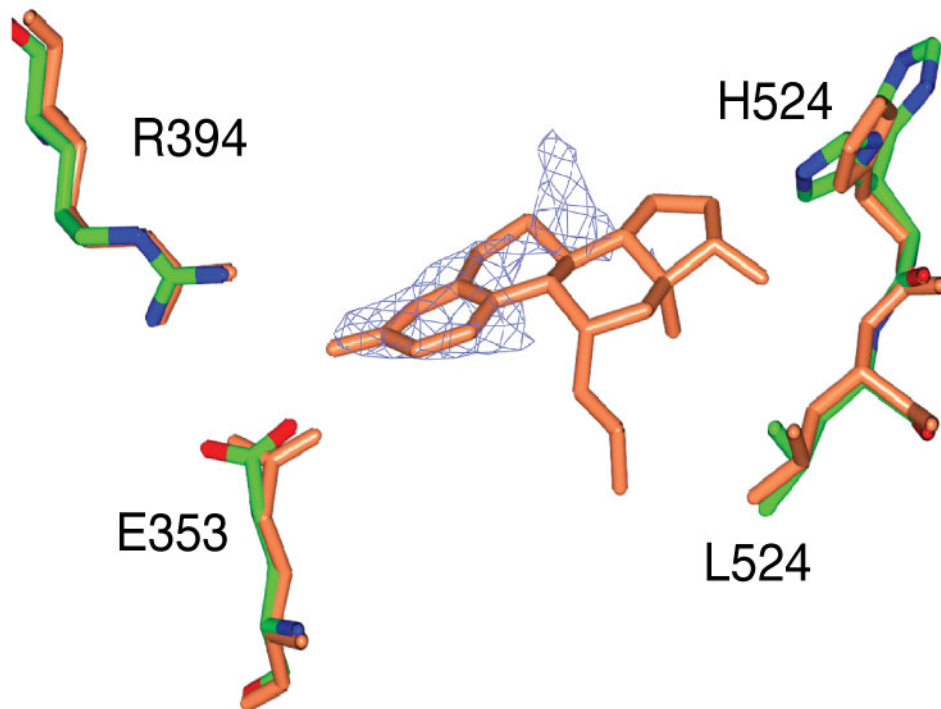
Geoffrey Greene Tel (773)702-6964, ggreene@uchicago.edu



Raloxifene (2)

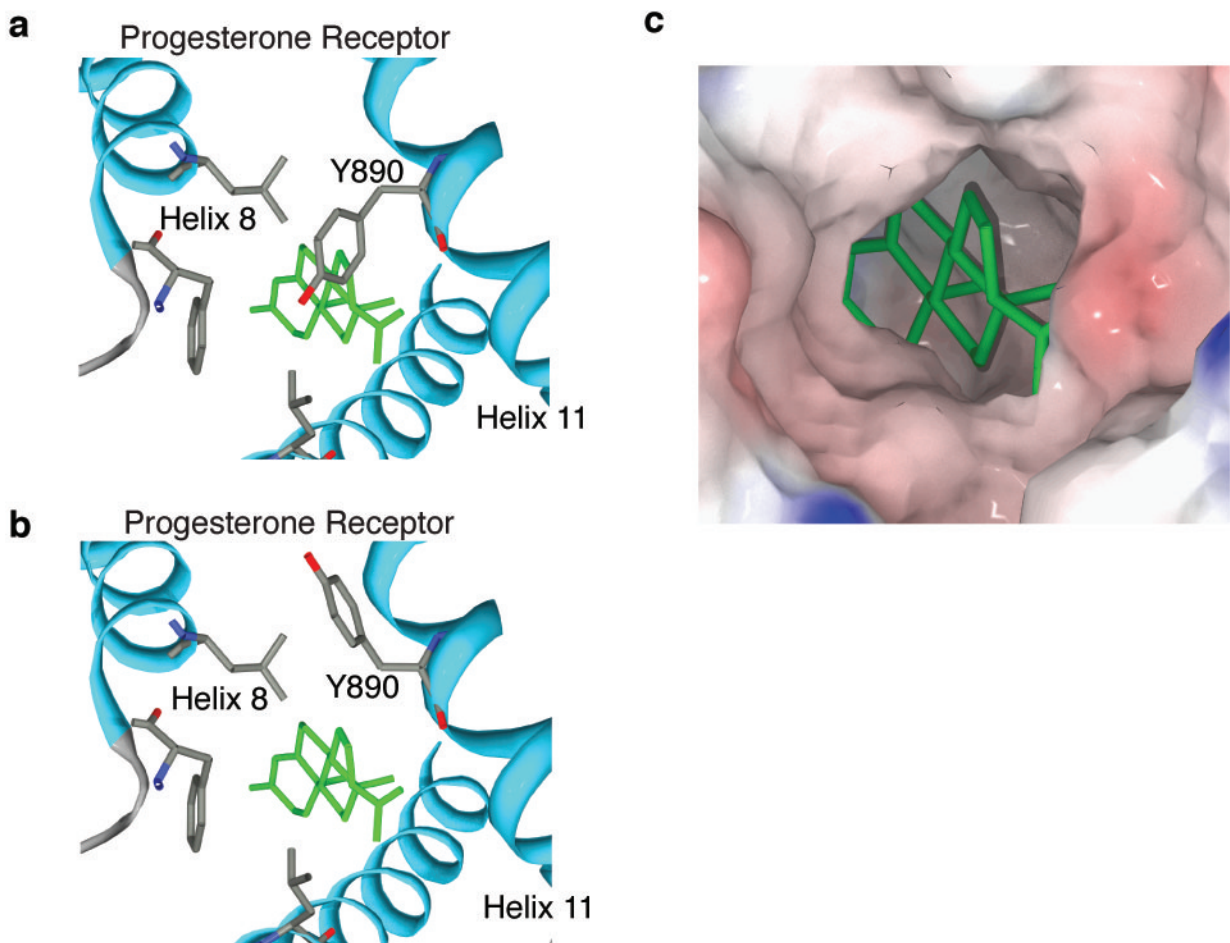
SI Fig 1. Electron Density of Raloxifene bound to ERα LBD Leu-536-Ser.

A 2Fo-Fc map is contoured at 1.8 σ .



SI Fig 2. Electron Density in the Ligand-Binding Pocket of the Apo ER LBD Structure.

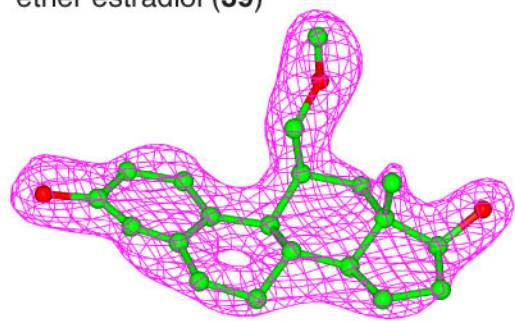
The structure of the mutant receptor displayed electron density that remained during refinement, shown as a 2Fo-Fc map and contoured at 1σ . Selected amino acids in the apo ER α ligand binding pocket are shown as stick figures, with carbon atoms colored green. The structure of the methoxymethyl ether estradiol-bound ER α was superimposed, and is colored orange.



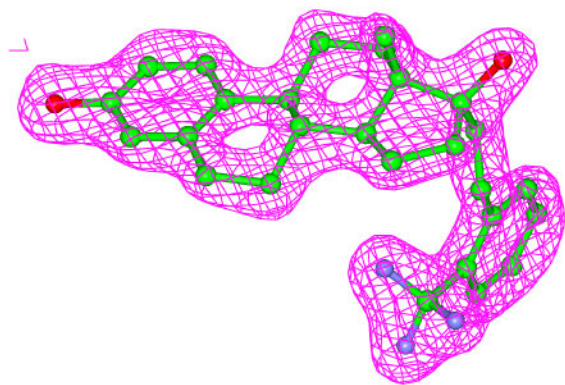
SI Fig 3. Molecular Modeling Suggests a Solvent Channel in the Progesterone Receptor.

- (a) The closed interface between helix 11 and the H7-8 loop is shown for the progesterone receptor (PDB code: 1A28), highlighting the structural conservation of this region. The ribbon trace is colored blue, and the progesterone ligand is colored green.
- (b) The Richardson rotamer library was used to model the most common positions for PR Tyr-890, revealing a common conformation that opens the conserved solvent channel.
- (c) The modeled solvent channel in PR is shown by an electrostatic rendering of the receptor, with the ligand shown as a green stick figure.

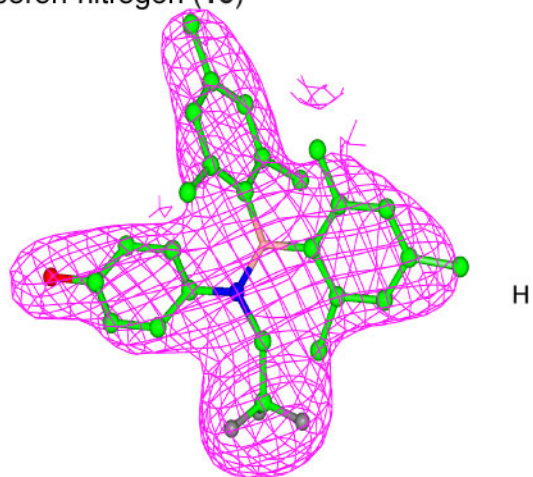
a ether estradiol (39)



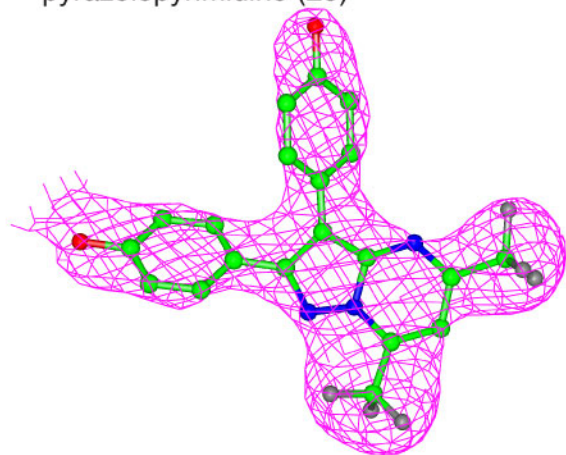
b phenylvinyl estradiol (40)



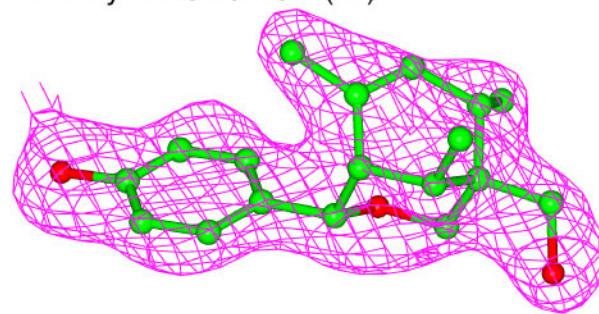
c boron-nitrogen (19)



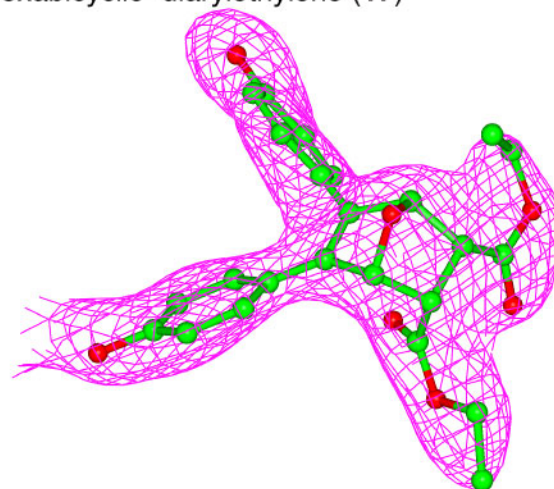
d pyrazolopyrimidine (20)



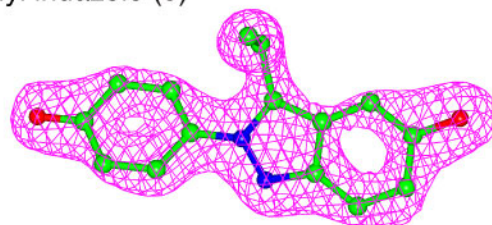
e oxabicyclic- OBCP-3M (36)



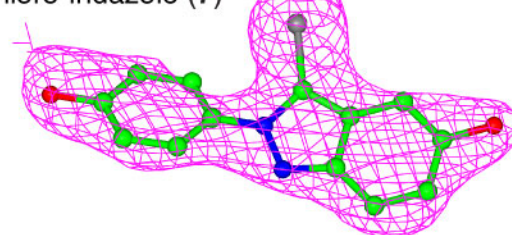
f oxabicyclic- diarylethylene (17)



g ethyl indazole (9)



h chloro-indazole (7)

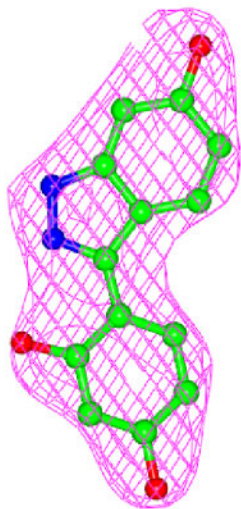


i genistein (6)

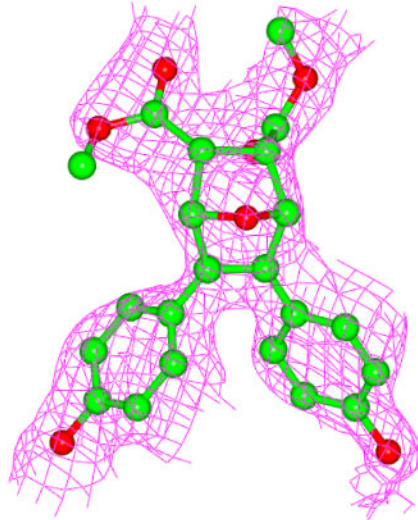


SI Fig 4. Structures of ligands bound to the ERα LBD

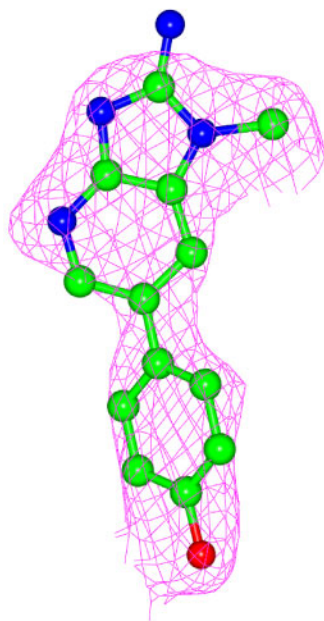
The indicated ligands were co-crystallized with the ERα LBD Tyr-537-Ser. Shown are the refined positions of the ligands in electron density (2Fo-Fc maps) contoured at 1.25 σ . Oxygen is colored red, nitrogen blue, boron pink, and halogens are light gray.



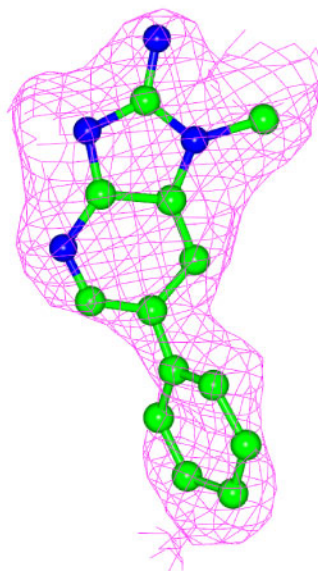
Indazolyphenol (42)



Dimethyl Oxabicyclic (43)



4OH-PhIP (44)

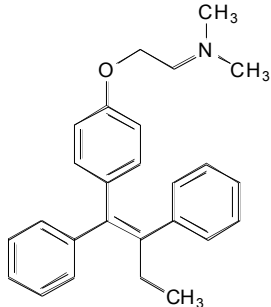
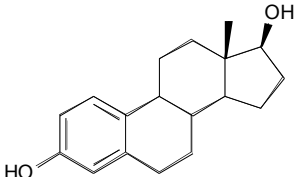
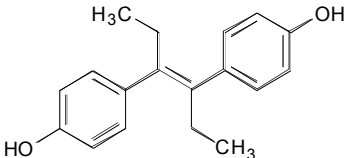
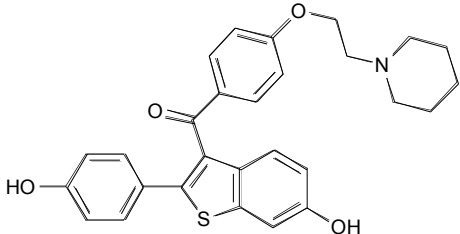


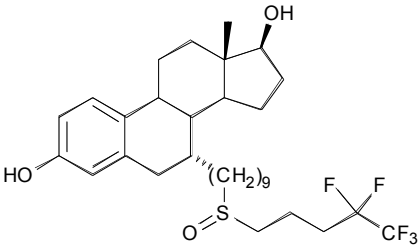
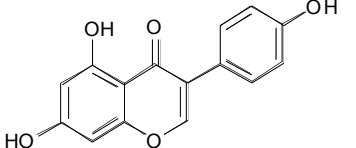
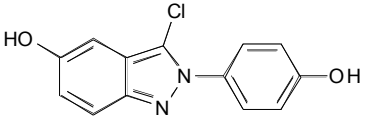
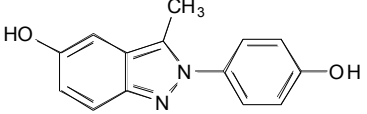
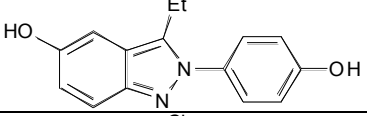
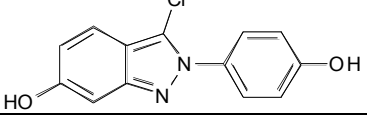
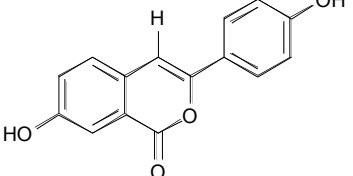
PhIP (45)

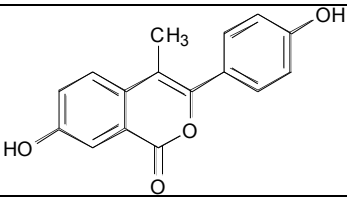
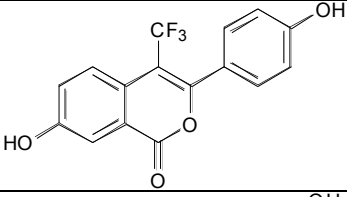
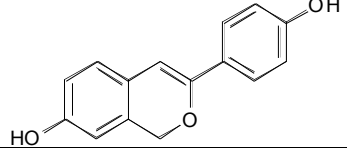
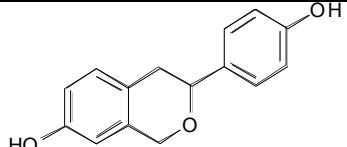
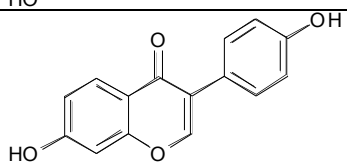
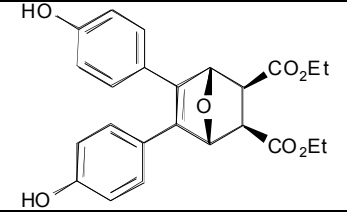
SI Fig 5. Electron density maps of ligands soaked into apo ER

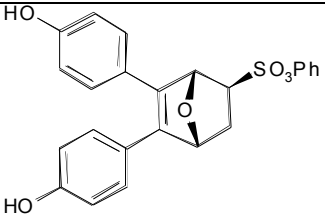
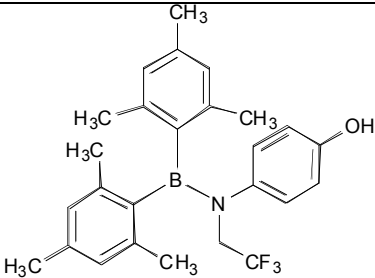
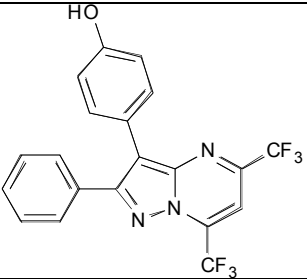
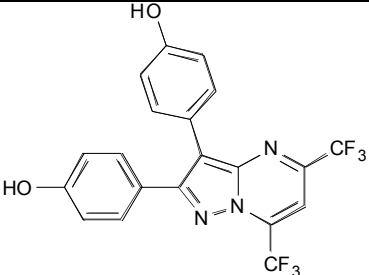
The indicated ligands were soaked into the apo-ER α crystals. Shown are the ligands docked into a 2Fo-Fc electron density map, contoured at 1.5 σ .

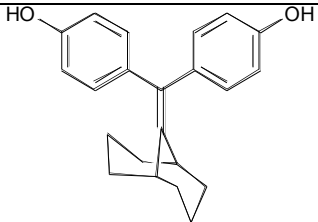
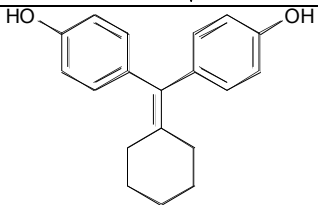
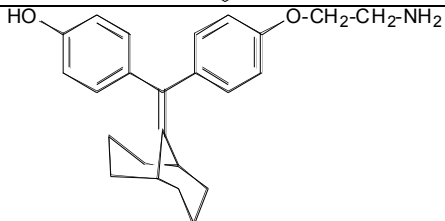
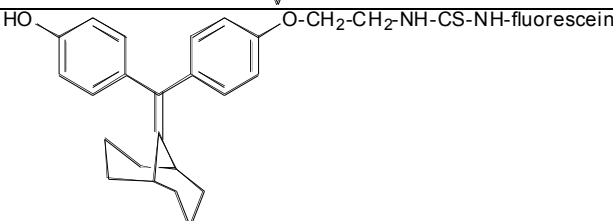
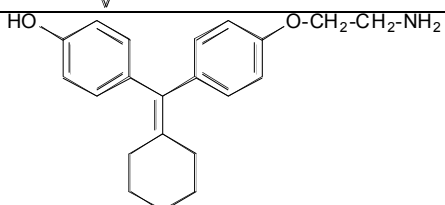
Supplemental Table 1. Chemical Structures

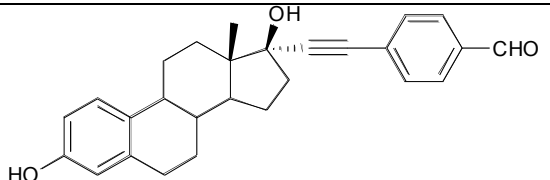
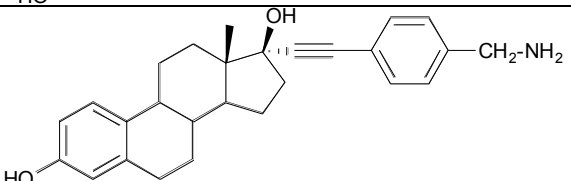
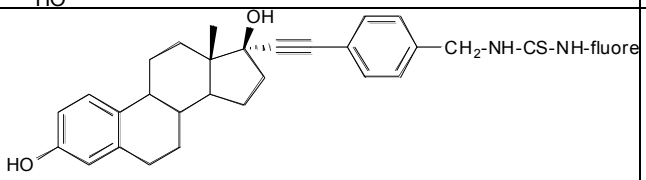
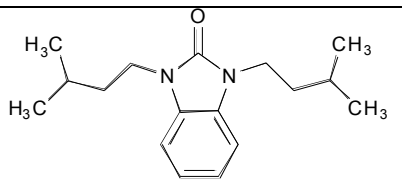
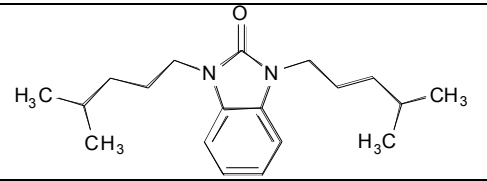
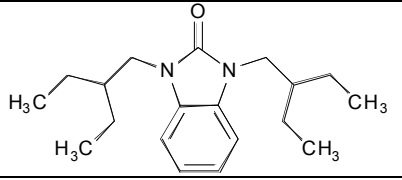
Name/Number	Structure	Reference	Characterization/purity for new compounds
<p>1</p> <p>Tamoxifen</p>		<p>Obtained from Sigma, >99% purity, catalog # T5648</p>	<p>Commercially Available</p>
<p>2</p> <p>Estradiol</p>		<p>Obtained from Sigma, >98% purity, catalog # E8875</p>	<p>Commercially Available</p>
<p>3</p> <p>Diethylstilbesterol</p>		<p>PDB code: 3ERD</p>	
<p>4</p> <p>Raloxifene</p>		<p>Obtained from Sigma, >99% purity, catalog #</p>	<p>Commercially Available</p>

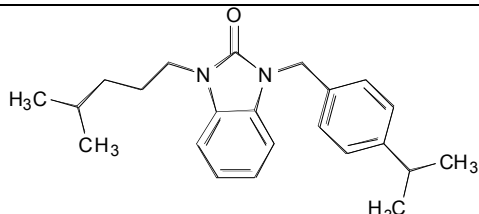
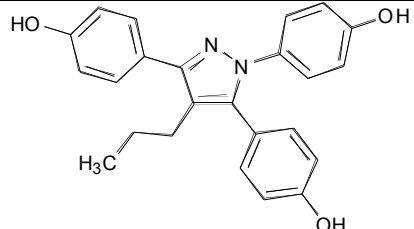
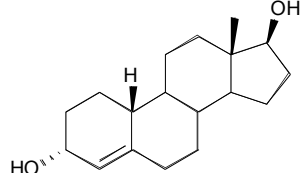
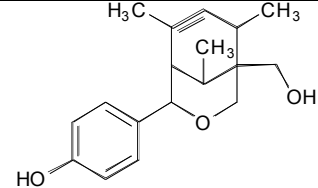
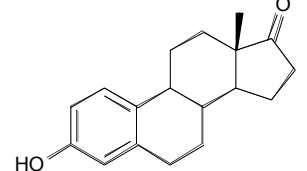
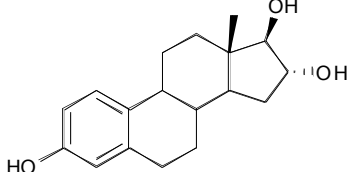
<p>5</p> <p>ICI 182,780</p>		<p>Obtained from Tocris (Ellisville, MO), >99% purity, catalog #1047</p>	<p>Commercially Available</p>
<p>6</p> <p>Genistein</p>		<p>Obtained from Sigma (St Louis, MO), ≥98% purity, catalog # C6649</p>	<p>Commercially Available</p>
<p>7</p> <p>Chloro-indazole</p>		<p>Reference 1 Compound 12c</p>	<p>Known; full characterization given in our prior publication</p>
<p>8</p> <p>MD 193</p>		<p>Reference 1 Compound 19b</p>	<p>Known; full characterization given in our prior publication</p>
<p>9</p> <p>Ethyl-indazole</p>		<p>Reference 1 Compound 16b</p>	<p>Known; full characterization given in our prior publication</p>
<p>10</p> <p>MD 202</p>		<p>Reference 1 Compound 30</p>	<p>Known; full characterization given in our prior publication</p>
<p>11</p> <p>MD 242</p>		<p>Reference 2 Compound 13b</p>	<p>Known; full characterization given in our prior publication</p>

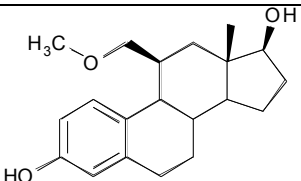
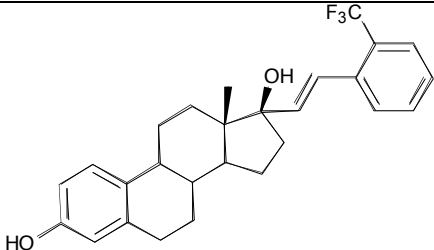
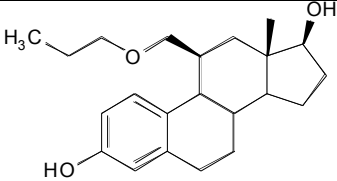
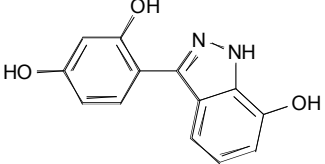
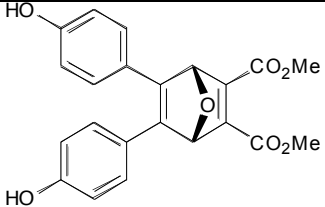
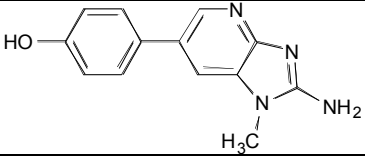
12 MD 257		Reference 2 Compound 16b	Known; full characterization given in our prior publication
13 MD 272		Reference 2 Compound 20b	Known; full characterization given in our prior publication
14 MD 266		Reference 2 Compound 25	Known; full characterization given in our prior publication
15 MD 271		Reference 2 Compound 23	Known; full characterization given in our prior publication
16 Daidzein		Obtained from Sigma (St Louis, MO), ≥98% purity, catalog # D7802	Commercially Available
17 diethyl oxabicyclic		Reference 3 Compound 12d	Known; full characterization given in our prior publication

<p>18</p> <p>HZ 3-11 04A</p>		<p>Reference 3</p> <p>Compound 12a</p>	<p>Known; full characterization given in our prior publication</p>
<p>19</p> <p>HZ 6-21-4</p>		<p>Reference 4</p> <p>Compound 9e</p>	<p>Known; full characterization given in our prior publication</p>
<p>20</p> <p>Pyrazolo-pyrimidine</p>		<p>Reference 5</p> <p>Compound 24b</p>	<p>Known; full characterization given in our prior publication</p>
<p>21</p> <p>DC-II-296A</p>		<p>Reference 5</p> <p>Compound 24c</p>	<p>Known; full characterization given in our prior publication</p>

<p>22</p> <p>KSH-6-19</p>		<p>Reference 6 Compound 8</p>	<p>Known; full characterization given in our prior publication</p>
<p>23</p> <p>Cyclofenil</p>		<p>Obtained from Sigma (St Louis, MO), ≥98% purity, catalog # C3490</p>	<p>Commercially Available</p>
<p>24</p> <p>KSH-5-19</p>		<p>New</p>	<p>See Characterization below for COMPOUND 24</p>
<p>25</p> <p>KSH-5-40</p>		<p>New</p>	<p>See Characterization below for COMPOUND 25</p>
<p>26</p> <p>KSH</p>		<p>New</p>	<p>See Characterization below for COMPOUND 26</p>

<p>27</p> <p>KSH-1-182</p>		<p>Reference 7 [Supporting Information] Co(pound 2a)</p>	<p>Known; but see also Characterization below for COMPOUND 27</p>
<p>28</p>		<p>New</p>	<p>See Characterization below for COMPOUND 28</p>
<p>29</p> <p>KSH-5-42-2</p>		<p>New</p>	<p>See Characterization below for COMPOUND 29</p>
<p>30</p> <p>TWM 1.17</p>		<p>New</p>	<p>See Characterization below for COMPOUND 30</p>
<p>31</p> <p>TWM 1.21</p>		<p>New</p>	<p>See Characterization below for COMPOUND 31</p>
<p>32</p> <p>TWM 1.95</p>		<p>New</p>	<p>See Characterization below for COMPOUND 32</p>

33 TWM 1.41		New	See Characterization below for COMPOUND 33
34 PPT		Obtained from Tocris (Ellisville, MO), >99% purity, catalog # 1426	Commercially Available
35 Estren		Obtained from EMD- Calbiochem, > 98% purity, Catalog # 330160	Commercially Available
36 OBCP-3M		Reference 8	Spectral data match reference
37 Estrone		Obtained from Sigma, >99% purity, catalog # E9750	Commercially Available
38 Estriol		Obtained from Sigma, >98% purity, catalog # 285803	Commercially Available

39 11 β -(1,1 _{ether}) estradiol		Reference 9 Compound 43	Known; full characterization given in our prior publication
40 TFMPV-Estradiol		Reference 10	Spectral data match reference
41 11 β -(1,3 _{ether}) estradiol		Reference 9 Compound 47	Known; full characterization given in our prior publication
42 Indazolyl phenol		Reference 11 Compound 6	Spectral data match reference
43 dimethyl oxabicyclic		Reference 3 Compound 12e	Known; full characterization given in our prior publication
44 4-OH PhIP		Reference 12	Spectral data match reference

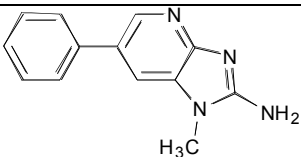
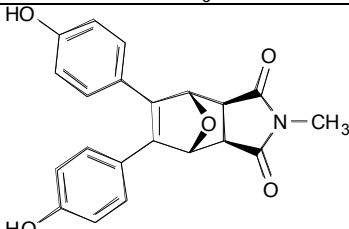
<p>45</p> <p>PhIP</p>		Reference 12	Spectral data match reference
<p>46</p> <p>Pyrrolidinedione oxabicyclic</p>		Reference 3 Compound 12f	Known; full characterization given in our prior publication

TABLE 2 REFERENCES:

1. De Angelis M, Stossi F, Carlson KA, Katzenellenbogen BS, Katzenellenbogen JA. Indazole Estrogens: Highly Selective Ligands for the Estrogen Receptor β . *J. Med. Chem.* **2005**, 48, 1132-1144.
2. De Angelis M, Stossi F, Waibel M, Katzenellenbogen BS, Katzenellenbogen JA. Isocoumarins as Estrogen Receptor Beta Selective Ligands: Isomers of Isoflavone Phytoestrogens and Their Metabolites. *Bioorg. Med. Chem.* **2005**, 13, 6529-6542.
3. Zhou HB, Comninou JS, Stossi F, Katzenellenbogen BS, Katzenellenbogen JA. Synthesis and Evaluation of Estrogen Receptor Ligands with Bridged Oxabicyclic Cores Containing a Diarylethylene Motif: Estrogen Antagonists of Unusual Structure. *J. Med. Chem.* **2005**, 48, 7261-7274.
4. Zhou HB, Nettles KW, Bruning JB, Kim Y, Joachimiak A, Sharma S, Carlson KE, Stossi F, Katzenellenbogen BS, Greene GL, Katzenellenbogen JA. Elemental Isomerism: A Boron-Nitrogen Surrogate for a Carbon-Carbon Double Bond Increases the Chemical Diversity of Estrogen Receptor Ligands. *Chem. Biol.*, **2007**, 14, 659-669.
5. Compton DR, Sheng S, Carlson KE, Rebacz NA, Lee IY, Katzenellenbogen BS, and Katzenellenbogen JA. Pyrazolo[1,5-a]Pyrimidines: Estrogen Receptor Ligands Possessing Estrogen Receptor β Antagonist Activity. *J Med. Chem.* **2004**, 47, 5872-5893.

6. Muthyala RS, Sheng S, Carlson KE, Katzenellenbogen BS, and Katzenellenbogen JA. Bridged Bicyclic Cores Containing a 1,1-Diarylethylene Motif Are High-Affinity Subtype-Selective Ligands for the Estrogen Receptor. *J Med. Chem* **2003**, 46, 1589-1602.
7. Kim SH, and Katzenellenbogen JA. Hormone-PAMAM Dendrimer Conjugates: Polymer Dynamics and Tether Structure Affect Ligand Access to Receptors. *Angew. Chemie* **2006**, 45, 7243-7248. [Supporting Information]
8. Hsieh RW, Rajan SS, Sharma SK, Guo Y, DeSombre ER, Mrksich M, Greene GL. Identification of ligands with bicyclic scaffolds provides insights into mechanisms of estrogen receptor subtype selectivity. *J Biol Chem.* **2006**, 281(26):17909-19.
9. Zhang JX, Labaree DC, Hochberg RB. Nonpolar and short side chain groups at C-11beta of estradiol result in antiestrogens. *J Med Chem.* **2005**, 48(5):1428-47.
10. Lee C-Y and Hanson RN. Solid phase synthesis of 17 α -E/Z-(X-phenyl)vinyl estradiols using the Stille coupling reaction. *Tetrahedron.* **2000**, 56:1623–1629.
11. Krishnan R, Lang, SA Jr., Lin YI, Wilkinson, RG. Reaction of hydroxybenzophenone with hydrazine. *J. Heterocyclic Chem.*, **1988**, 25, 447-452.
12. Knize MG and Felton JS. The synthesis of the cooked-beef mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and its 3-methyl isomer. *Heterocycles.* **1986**, 24(7):1815-1819.

Supplemental Table 2. Crystallographic Summary

	Dimethyl Oxabicyclic (43)	Genistein (6)	Indazolyl Phenol (42)	4OH-PhIP (44)	PhIP (45)
Data collection					
Space group	P1211	P1211	P1211	P1211	P1211
Cell dimensions		pyrazolopyrimidine			
<i>a, b, c</i> (Å)	55.99,84.00,58.72	55.99,84.00,58.72	55.99,84.00,58.72	56.08,84.04,58.67	55.97,83.70,58.43
α, β, γ (°)	90.00,108.85,90.00	90.00,108.85,90.00	90.00,108.85,90.00	90.00,108.85,90.00	90.00,108.70,90.00
Resolution (Å)	10.0-2.00(2.07-2.00)*	10.0-2.00(2.07-2.00)*	10.0-2.00(2.07-2.00)*	15.0-1.85(1.90-1.85)*	12.0-2.3(2.35-2.30)*
<i>R</i> _{merge}	6.2(15.6)	4.9	53.5)	6.4(28.3)	12.0(24.1)
<i>I</i> / σ <i>I</i>	18.7(10.7)	20	2.4)	18.8(3.7)	9.6(3.7)
Completeness (%)	89.4(92.2)	97.0(81.0)	92.5(67.5)	95.1(71.2)	88.8(86.9)
Redundancy	4.0(4.0)	3.1(2.4)	6.2(3.1)	4.3(2.5)	3.3(3.0)
Refinement					
Resolution (Å)	10.0-2.0	19.9-1.85	15-2.6	15-1.85	12-2.3
No. reflections					
<i>R</i> _{work} / <i>R</i> _{free}	21.4/26.2	21.1/26.4	19.5/24.9	16.9/22.5	27.3/29.6
No. atoms					
Protein	4021	3905	3985	3987	3960
Ligand/ion	58	40	36	36	34
Water	159	70	41	359	5
<i>B</i> -factors					
Protein	20.95	48.92	42.56	27.05	31.70
Ligand/ion	52.91	28.31	64.34	82.05	46.15
Water	40.21	27.10	63.72	11.45	11.50
R.m.s. deviations					
Bond lengths (Å)	0.014	0.010	0.016	0.014	0.018
Bond angles (°)	1.546	1.278	1.559	1.422	1.540

Data for each structure collected from a single crystal. *Highest-resolution shell is shown in parentheses.

[AU: Equations defining various R values are standard and hence are no longer defined in the footnotes.]

[AU: Ramachandran statistics should be in methods section at the end of the refinement sub-section.]

[AU: Wavelength of data collection, temperature, beamline should all be in methods section.]

Supplemental Table 1. Crystallographic Summary, continued

	apo	Ether estradiol (39)	Ethyl Indazole (9)	Chloro- Indazole (7)	Diethyl Oxabicyclic (17)
Data collection					
Space group	P 1 21 1	P 1 21 1	P 1 21 1	P 1 21 1	P 1 21 1
Cell dimensions					
<i>a</i> , <i>b</i> , <i>c</i> (Å)	54.37,80.90,58.24	58.17,83.71,56.01	56.05,84.27,58.21	55.83,83.08,58.30	55.77,81.72,58.31
α , β , γ (°)	90.00, 109.68, 90.00	90.00, 108.45, 90.00	90.00, 108.48, 90.00	90.00, 109.13, 90.00	90.00, 109.90, 90.00
Resolution (Å)	30.0-2.10 (2.15-2.10) *	55.0-2.15 (2.23-2.15) *	50.0-2.35 (2.43-2.35) *	50.0-1.86 (1.93-1.86) *	20.0-2.70 (2.80-2.70) *
<i>R</i> _{merge}	6.5(39.0)	11.8(39.0)	9.7(41.5)	9.8(68.8)	11.3(54.6)
<i>I</i> / σ <i>I</i>	14.0(2.3)	8.9(1.8)	11.2(1.8)	26.9(1.9)	21.6(3.5)
Completeness (%)	94.5(94.7)	98.7(95.7)	95.7(93.6)	98.3(95.9)	97.8(98.7)
Redundancy	5.5(5.3)	3.7(3.1)	3.8(3.2)	3.6(2.8)	3.5(3.4)
Refinement					
Resolution (Å)	30-2.10	55-2.15	50-2.39	19.54-1.89	19.73-2.7
No. reflections					
<i>R</i> _{work} / <i>R</i> _{free}	21.7/27.2	19.2/23.96	23.8/29.6	22.39/26.95	20.5/29.10
No. atoms					
Protein	3906	3993	3982	4045	3880
Ligand/ion	0	46	36	38	62
Water	30	134	216	16	3
<i>B</i> -factors					
Protein	33.30	20.11	29.99	32.02	16.49
Ligand/ion		13.98	98.56	19.74	28.83
Water	31.12	23.60	26.61	32.93	18.89
R.m.s deviations					
Bond lengths (Å)	0.009	0.011	0.021	0.009	0.018
Bond angles (°)	1.157	1.149	1.993	1.051	1.920

Data for each structure collected from a single crystal. *Highest-resolution shell is shown in parentheses.

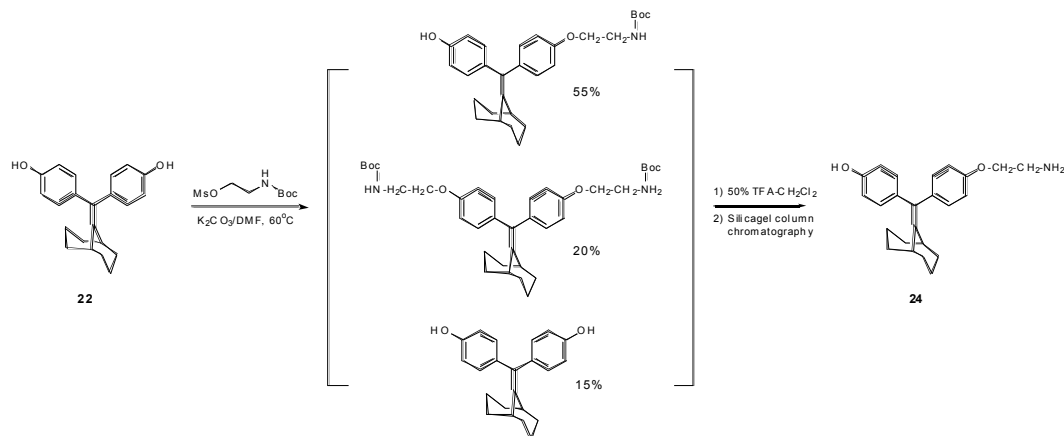
[AU: Equations defining various R values are standard and hence are no longer defined in the footnotes.]

[AU: Ramachandran statistics should be in methods section at the end of the refinement sub-section.]

[AU: Wavelength of data collection, temperature, beamline should all be in methods section.]

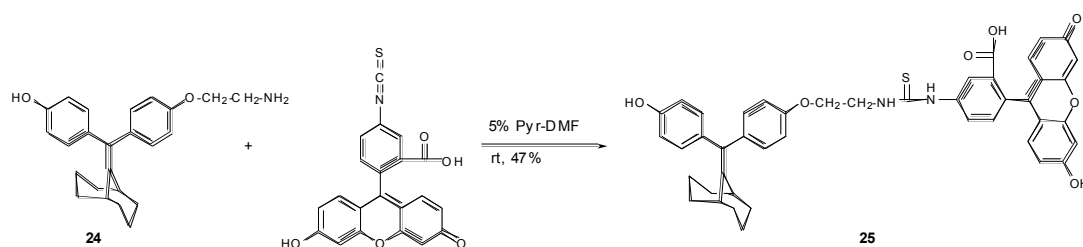
SUPPLEMENTAL METHODS

SYNTHETIC ROUTES TO AND SPECTROSCOPIC CHARACTERIZATION OF NEW COMPOUNDS:



COMPOUND 24:

The mixture of 4,4'-((1s,5s)-bicyclo[3.3.1]nonan-9-ylidene)methylendiphenol **22** (320 mg, 1.0 mmol), (t-butyloxycarbonyl)aminoethoxy methanesulfonate (239 mg, 1.0 mmol), and K_2CO_3 (200 mg) in DMF (5.0 ml) was stirred for 2 hrs at 60 °C. To this reaction mixture was added water (20 ml) and extracted with ethyl acetate (10 ml x 3). Ethyl acetate dried over $MgSO_4$ was evaporated to afford the mixture of mono-, dialkylated compounds, and starting material. This mixture was treated with 50% TFA- CH_2Cl_2 and loaded onto silicagel for chromatography after concentrating 50% TFA- CH_2Cl_2 solvent, washing the residue with water, and drying it. Elution with a ethyl acetate gave a starting material **22**. Continuous elution with a 10% MeOH- $CHCl_3$ provided a 4-((4-(2-aminoethoxy)phenyl)((1s,5s)-bicyclo[3.3.1]nonan-9-ylidene)methyl)phenol **24** (200 mg, 55%) as a colorless powder. mp 134-136 °C (dec); 1H NMR (500 MHz, Methanol- d_4) δ 1.55-1.62 (m, 2H), 1.71-1.87 (m, 8H), 2.00-2.12 (m, 2H), 2.62 (s, 1H), 2.69 (s, 1H), 3.34 (t, 2H, $J = 5.0$ Hz), 4.20 (t, 2H, $J = 5.0$ Hz), 6.68 (d, 2H, $J = 5.0$ Hz), 6.92 (d, 4H, $J = 5.0$ Hz), 7.07 (d, 2H, $J = 5.0$ Hz); ^{13}C NMR (126 MHz, Methanol- d_4) δ 21.51, 33.52, 33.56, 34.23, 34.33, 39.14, 64.05, 113.96, 114.51, 130.08, 130.27, 131.04, 134.50, 137.15, 144.35, 155.62, 156.57; HRMS (ESI) m/z calcd for $C_{24}H_{30}NO_2$ ($M^+ + 1$) 364.2277, found 364.2272, ; Elemental analysis: Calcd for $C_{24}H_{29}NO_2$ 1/2 H_2O C 77.38, H 7.85, N 3.76, found C 74.66, H 7.56, N 4.16.



COMPOUND 25:

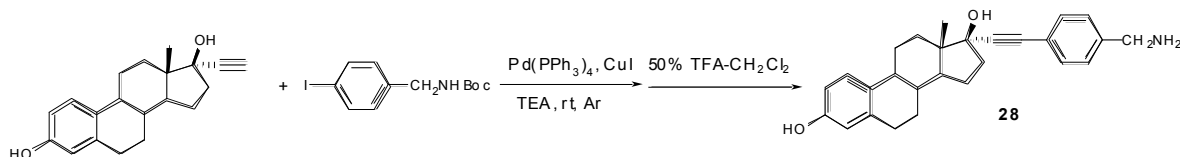
The mixture of 4-((4-(2-aminoethoxy)phenyl)((1s,5s)-bicyclo[3.3.1]nonan-9-ylidene)methyl)phenol (**24**, 36 mg, 0.1 mmol) and FITC (39 mg, 0.1 mmol) in 5% pyridine-DMF (1 ml) was stirred for 4 hrs at rt. Yellow solid was precipitated by addition of 1 N HCl (4 ml) to the mixture solution. The solid was collected by filtration and rinsed several times with 1N HCl solution. After drying, the mixture was loaded on silicagel PLC (1 mm, 20 x 20 cm). 10% MeOH-CHCl₃ was used as an eluent. Collection of yellow band (R_f = 0.5) by scratching out and extraction with same solution as an eluent gave a yellowish powder (35 mg, 47%). ¹H NMR (500 MHz, Methanol-d₄) δ 1.55-1.66 (m, 2H), 1.71-1.89 (m, 8H), 1.98-2.14 (m, 2H), 2.65 (s, 1H), 2.70 (s, 1H), 3.98 (m, 2H), 4.21 (t, 2H, J = 5.0 Hz), 6.62 (d, 2H, J = 5.0 Hz), 6.63-6.86 (m, 4H), 6.93 (d, 4H, J = 5.0 Hz), 6.93-7.06 (m, 2H), 7.09 (d, 2H, J = 5.0 Hz); 7.14 (d, 1H, J = 8.0 Hz), 7.85 (d, 1H, J = 8.0 Hz), 8.29 (s, 1H); HRMS (ESI) m/z calcd for C₄₅H₄₁N₂O₇S (M⁺+1) 753.2634, found 753.2648

COMPOUND 26:

4-((4-(2-Aminoethoxy)phenyl)(cyclohexylidene)methyl)phenol **26** was synthesized from the 4,4'-(cyclohexylidenemethylene)diphenol **23** (280 mg, 1.0 mmol), (t-butylloxycarbonyl)aminoethoxy methanesulfonate (239 mg, 1.0 mmol), and K₂CO₃ (200 mg) as described in the synthesis of compound **24**. mp 152-154 °C (dec); ¹H NMR (500 MHz, Methanol-d₄) δ 1.51-1.65 (m, 6H), 2.18-2.29 (m, 4H), 3.12 (t, 2H, J = 5.0 Hz), 4.05 (t, 2H, J = 5.0 Hz), 6.68 (d, 2H, J = 5.0 Hz), 6.86 (d, 2H, J = 5.0 Hz), 6.87 (d, 2H, J = 5.0 Hz), 6.99 (d, 2H, J = 5.0 Hz); ¹³C NMR (125 MHz, Methanol-d₄) δ 26.80, 28.59, 32.30, 40.05, 66.97, 113.88, 114.46, 130.65, 130.78, 134.11, 134.65, 137.19, 137.85, 155.56, 156.55; MS (ESI) m/z 324.4 (M⁺+1); HRMS (ESI) m/z calcd for C₂₁H₂₆NO₂ (M⁺+1) 324.1963, found 324.1964; Elemental analysis: Calcd for C₂₁H₂₅NO₂ 0.8H₂O C 74.66, H 7.46, N 4.15, found C 74.66, H 7.56, N 4.16

COMPOUND 27:

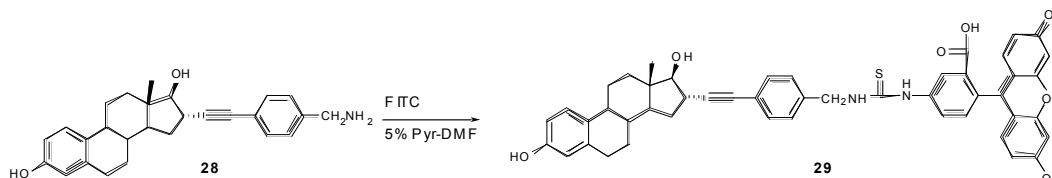
mp 138-140 °C (dec.); ¹H NMR (400 MHz, CDCl₃) δ 0.93 (s, 3H), 1.25-2.81 (m, 15H), 6.47 (d, 1H, J = 2.4 Hz), 6.53 (dd, 1H, J = 2.4 Hz, J = 8.0 Hz), 7.09 (d, 1H, J = 8.0 Hz), 7.60 (d, 2H, J = 10.5 Hz), 7.87 (d, 2H, J = 10.5 Hz), 9.97 (s, 1H, aldehyde); ¹³C NMR (125 MHz, CDCl₃) δ 13.09, 23.15, 26.64, 27.40, 29.82, 33.33, 39.26, 39.65, 43.85, 47.98, 50.15, 66.10, 85.48, 97.21, 110.00, 112.94, 115.48, 126.80, 129.79, 132.43, 132.63, 135.68, 138.47, 191.78; HRMS (EI) m/z calcd for C₂₇H₂₈O₃ (M⁺) 400.203845, found 400.203872; Elemental analysis: Calcd for C₂₇H₂₈O₃ 1.0 H₂O C 77.48, H 6.74, found C 77.80, H 6.74.



COMPOUND 28:

The mixture of 4-(t-butylloxycarbonylaminomethyl)iodobenzene (167 mg, 0.5 mmol), tetrakis(triphenylphosphine)palladium (5 mole %), and CuI (5 mole %) in triethylamine (20 ml) was added 17α-ethynylestradiol (148 mg, 0.5 mmol) under argon atmosphere at rt. This reaction mixture was stirred for 6 hrs at the same temperature. The solvent was evaporated under reduced pressure. The residue was treated with 50% TFA-CH₂Cl₂ and subsequently loaded on the silicagel column chromatography. Elution with ethyl acetate gave unreacted 17α-ethynylestradiol. Elution with a 5% MeOH-CHCl₃ afforded (13S,16S,17S)-16-((4-(aminomethyl)phenyl)ethynyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-

cyclopenta[a]phenanthrene-3,17-diol (150 mg, 75%) **28** as an colorless powder. mp 143-145 °C (dec.); ¹H NMR (500 MHz, Methanol-d₄) δ 0.89 (s, 3H), 1.25-2.81 (m, 15H), 3.75 (s, 2H), 6.46 (d, 1H, J = 2.4 Hz), 6.54 (dd, 1H, J = 2.4 Hz, J = 8.0 Hz), 7.06 (d, 1H, J = 8.0 Hz), 7.27 (d, 2H, J = 10.5 Hz), 7.37 (d, 2H, J = 10.5 Hz); ¹³C NMR (125 MHz, CDCl₃) δ 13.30, 22.69, 26.59, 27.45, 29.49, 33.21, 38.78, 39.95, 43.98, 45.11, 49.96, 60.36, 79.66, 85.05, 93.14, 112.62, 114.93, 122.13, 126.14, 127.41, 131.29, 131.49, 137.64, 142.19, 154.79; HRMS (ESI) m/z calcd for C₂₇H₃₂NO₂ (M⁺) 402.2433, found 402.2426; Elemental analysis: Calcd for C₂₇H₃₁NO₂ · 1.5H₂O C 75.67, H 7.29, N 3.27; found C 75.28, H 7.31, N 3.24

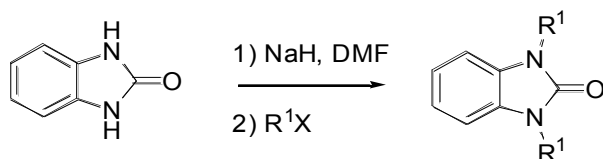


COMPOUND 29:

(13S,16S,17S)-16-((4-[4'-Thioureido-6-hydroxy-3-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol **29** was synthesized from (13S,16S,17S)-16-((4-(aminomethyl)phenyl)ethynyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol **28** (40 mg, 0.1 mmol) and FITC (39 mg, 0.1 mmol) in 5% pyridine-DMF (1 ml) as described in the synthesis of **25**. ¹H NMR (500 MHz, Methanol-d₄) δ 0.92 (s, 3H), 4.63 (s, 2H, Benzyl CH₂), 6.47 (d, 1H, J = 2.4 Hz), 6.53 (dd, 1H, J = 2.4 Hz, J = 10.0 Hz), 6.59 (d, 2H, J = 8.0 Hz), 6.69 (s, 2H), 6.82 (s, 2H), 7.10 (d, 1H, J = 10.0 Hz), 7.33 (d, 1H, J = 8.0 Hz), 7.37 (d, 2H, J = 8.0 Hz), 7.42 (d, 2H, J = 8.0 Hz), 8.14 (d, 2H, J = 8.0 Hz), 8.47 (s, 1H); HRMS (ESI) m/z calcd for C₄₈H₄₃N₂O₇S (M⁺+1) 791.2791, found 791.2789;

GENERAL PROCEDURE FOR SYNTHESIS OF SYMMETRICALLY SUBSTITUTED BENZIMIDAZOLONES:

In a glove bag filled with N₂, a suspension of sodium hydride (60% in mineral oil, Aldrich—2.2 equivalents) was weighed into a flame-dried three-neck flask. The flask was evacuated and back-filled with N₂ three times. DMF (Aldrich Biotech grade, < 0.005% water) was added to the flask, followed by 2-hydroxybenzimidazole (1.0 equivalents, Aldrich, 97%). The mixture was allowed to stir at ambient temperature for one-half hour. Alkyl halide (2.0 equivalents) was added to the flask and stirred at ambient temperature (alkyl bromide) or 80 °C (alkyl chloride) overnight. The reaction was quenched by pouring into water and extracting three times with EtOAc. The combined organic extracts were dried over MgSO₄ and filtered by gravity filtration. The solvents were evaporated by rotary evaporation, and the crude product was dried under high vacuum.



COMPOUND 30:

1,3-Bis-(3-methylbutyl)-1,3-dihydrobenzimidazol-2-one. Benzimidazolone **30** was prepared according to the general procedure for the synthesis of symmetrically substituted benzimidazolones. Flash column chromatography (heptane:EtOAc, 6:1 v/v, R_f=0.35) afforded

the product as white crystals (360 mg, 35%): mp 49-51 °C; ¹H NMR (400 MHz, CDCl₃): δ 7.10-7.07/7.00-6.96 (AA'XX', 4H), 3.89 (m, 4H), 1.63 (m, 6H), 0.98 (d, 12H, *J* = 6.4 Hz); ¹³C NMR (100MHz, CDCl₃) δ 154.1, 129.4, 120.8, 107.5, 39.4, 37.0, 25.8, 22.4; LRMS (EI, 70 eV): 274.3 (M⁺, 100%), 217 (50%), 162 (49%); HRMS (EI): calcd. for C₁₇H₂₆N₂O, 274.2045; found, 274.2048; analysis (calcd., found for C₁₇H₂₆N₂O): C (74.41,74.56), H (9.55,9.90), N (10.21,10.16).

COMPOUND 31:

1,3-Bis-(4-methylpentyl)-1,3-dihydrobenzimidazol-2-one. Benzimidazolone **31** was prepared according to the general procedure for the synthesis of symmetrically substituted benzimidazolones. Flash column chromatography (hexanes:EtOAc, 3:1 v/v, R_f=0.44) afforded the product as a clear yellow oil (358 mg, 64%): ¹H NMR (400 MHz, CDCl₃): δ 7.11-7.07/7.01-6.96 (AA'XX', 4H), 3.86 (t, 4H, *J* = 7.32 Hz), 1.75 (m, 4H), 1.58 (nonet, 2H, *J* = 6.59 Hz), 1.26 (m, 4H), 0.88 (d, 12H, *J* = 6.59 Hz); ¹³C NMR (100 MHz, CDCl₃): δ 154.1, 129.4, 120.8, 107.5, 41.3, 35.8, 27.7, 26.3, 22.5; LRMS (EI, 70 eV): 302.3 (M⁺, 100%), 231.2 (41%), 218.2 (40%); HRMS (EI): calcd. for C₂₇H₃₀N₂O, 302.2358; found, 302.2351.

COMPOUND 32:

1,3-Bis-(2-ethylbutyl)-1,3-dihydrobenzimidazol-2-one. Benzimidazolone **32** was prepared according to the general procedure for the synthesis of benzimidazolones. Flash column chromatography (hexanes:EtOAc, 3:1 v/v, R_f=0.48) afforded the product as an off-white waxy solid (138 mg, 30%): mp 28-29 °C; ¹H NMR (400 MHz, CDCl₃): δ 7.08-7.06/6.99-6.97 (AA'XX', 4H), 3.77 (d, 4H, *J* = 7.57 Hz), 1.86 (septet, 2H, *J* = 6.39 Hz), 1.37 (quintet, 8H, *J* = 7.26 Hz), 0.92 (d, 12H, *J* = 7.45 Hz); ¹³C NMR (100 MHz, CDCl₃): δ 155.1, 130.1, 121.0, 108.0, 45.0, 40.0, 23.6, 10.9; LRMS (EI, 70 eV): 302.3 (M⁺, 68%), 231.2 (66%), 218.2 (100%); HRMS (EI): calcd. for C₁₉H₃₀N₂O, 302.2358; found, 302.2353; analysis (calcd., found for C₁₉H₃₀N₂O): C (75.45, 75.40), H (10.00, 10.37), N (9.26, 9.31).

COMPOUND 33:

1-(4-*i*-Propylbenzyl)-1,3-dihydrobenzimidazol-2-one. Sodium hydride (1.0 equivalent, Aldrich, 60% in mineral oil) was added to a dry 2-necked round-bottom flask, which was evacuated and charged with N₂ three times. DMF (Fisher Reagent Grade) was added to the flask, followed by 1-(1-methylethenyl)-1,3-dihydrobenzimidazol-2-one (1.0 equivalent, prepared as described by Meth-Cohn and Smith¹). This mixture was allowed to stir for thirty minutes. 4-Isopropylbenzyl bromide (1.0 equivalent) was added to the flask and stirred overnight at ambient temperature. The reaction was quenched by adding sulfuric acid (9 M, 4.0 equivalents). This solution was allowed to stir at ambient temperature overnight. The solution was poured into water and cooled. The resultant off-white powder was filtered and used without further purification. (470 mg, 88%): mp 170-171 °C; ¹H NMR (400 MHz, CDCl₃): δ 10.47 (s, 1H, NH), 7.29-7.27/7.19-7.17 (AA'XX', 4H), 7.15-6.91 (m, 4H), 5.08 (s, 2H), 2.88 (septuplet, 1H, *J*=6.84 Hz), 1.22 (d, 6H, *J*=7.08 Hz); ¹³C NMR (100 MHz, CDCl₃): δ 155.8, 148.3, 133.5, 130.2, 127.4, 126.8, 121.6, 121.3, 109.7, 108.5, 44.3, 33.8, 23.9; LRMS (EI, 70 eV): 266.2 (M⁺, 57%), 133.1 (100%); HRMS (EI): calcd for C₁₇H₁₈N₂O 266.1419, found 266.1418.

1-(4-*i*-Propylbenzyl)-3-(4-methylpentyl)-1,3-dihydrobenzimidazol-2-one. Sodium hydride (1.1 equivalents, Aldrich, 60% in mineral oil) was placed in a dry 2-necked round-bottom flask, which was evacuated and back-filled with N₂ three times. DMF (Fisher Reagent Grade) was added to the flask, followed by the previously prepared 1-(4-*i*-propylbenzyl)-1,3-

¹ Meth-Cohn, O.; Smith, D. I. N-bridged heterocycles. Part 5. α,ω-Bis-(2-oxobenzimidazolyl)alkanes and ethers as selective ligands for Group IA and IIA metals. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1, 261-270.

dihydrobenzimidazol-2-one (1.0 equivalent). The mixture was allowed to stir for thirty minutes, and 4-methylpentyl bromide (1.0 equivalent) was added to the flask. The reaction mixture was stirred overnight under an atmosphere of N₂ and was quenched by pouring into water and extracting three times with organic solvent (EtOAc). The combined organic extracts were dried over MgSO₄ and filtered by gravity filtration. The solvents were evaporated by rotary evaporation, and the crude product was dried under high vacuum. Preparative thin-layer chromatography (hexanes:EtOAc, 6:1 v/v, R_f=0.29) afforded the product as a yellow waxy solid (83 mg, 63%): mp 51-52 °C; ¹H NMR (400 MHz, CDCl₃): δ 7.26-7.24/7.17-7.15 (AA'XX', 4H), 7.08-6.98/6.92-6.90 (m, 4H), 5.04 (s, 2H), 3.89 (t, 2H, *J* = 7.50 Hz), 2.86, (septuplet, 1H, *J* = 6.93 Hz), 1.77 (m, 2H), 1.59 (septuplet, 1H, *J* = 6.69 Hz), 1.27 (m, 2H), 1.21 (d, 6H, *J* = 6.86 Hz), 0.88 (d, 6H, *J* = 6.65 Hz); ¹³C NMR (125 MHz, CDCl₃): δ 154.4, 148.3, 133.9, 129.6, 129.4, 127.6, 126.8, 121.1, 121.0, 108.3, 107.7, 44.6, 41.5, 35.9, 33.8, 27.8, 26.3, 24.0, 22.5; LRMS (EI, 70 eV): 350.3 (M⁺, 59%), 133.1 (100%); HRMS (EI): calcd. for C₂₃H₃₀N₂O, 350.2358; found, 350.2358.

